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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/214,124	03/17/99	LOPEZ LASTRA M	017753-109

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NGUYEN, D	
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Please find below and/or attached an Office communication concerning this application or proceeding.

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Office Action Summary	Application No.	Applicant(s)	
	09/214,124	LOPEZ LASTRA ET AL.	
	Examiner	Art Unit	
	Quang Nguyen	1632	

-- The MAILING DATE of this communication appears on the cover she t with the correspondenc address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 January 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *8-19,22,23 and 25-50* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8-19,22,23 and 25-50* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' amendment filed on January 05, 2001 in Paper No. 11 has been entered. Amended claims 8-19, 22-23 and newly added claims 25-50 are pending in the present application.

The text of those sections of Title 35 U.S.C. Code not included in this action can be found in a prior office action.

Response to Amendment

The rejection of claims 1, 2 and 8-24 under 35 U.S.C. 112, First paragraph for Written Description is withdrawn.

The rejection of claim 22 under 35 U.S.C. 102(e) as being anticipated by Hora et al. (U.S. Patent No. 5,997,856) is withdrawn in light of Applicants' amendment.

Claim Rejections - 35 USC § 112

Claims 22, 23 and 37 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention for the reasons already set forth in the previous Office Action in Paper No. 9 (pages 2-9).

Claims 22 and 23 are drawn to a method of preparing a pharmaceutical composition comprising as therapeutic or prophylactic agent, a vector,. a viral particle generated from a viral vector, a cell comprising a vector or a virus particle generated

from a viral vector of claim 8 in combination with a pharmaceutically acceptable vehicle, the same pharmaceutical composition wherein the composition comprises between 10^4 and 10^{14} pfu. Claim 37 is directed to a method of treating or preventing a genetic disease, a cancer or an infection disease, comprising the step of administering a therapeutically effective quantity of a vector according to claim 8, a viral particle according to claim 18 or a cell according to claim 19, to a patient requiring such a treatment.

The specification discloses the construction of mono- and dicistronic vector plasmids comprising the 5' non-translational leader of avian reticuloendotheliosis virus type A (REV-A) and characterizes the Internal Ribosomal Entry Segment (IRES) within said 5' non-translational leader. It appears that the minimal IRES sequence resides within a fragment (nucleotides 452-578) of the 5' leader. The specification further discloses the construction of series of retroviral vectors comprising the REV-A sequences containing the minimal IRES site. The retroviral vectors possess either Mo-MLV type LTRs (pREV HW vector series) or spleen necrosis virus (SNV) type LTRs (pMC vector series not disclosed). The infectious viral particles generated from these retroviral vectors were used to determine the viral titer and the expression of reporter genes (placental alkaline phosphatase and neo). The specification teaches that retroviral vectors comprising both a REV-A sequence (nucleotide fragments 265-578 or 452-578) and a conventional encapsidation region (Mo-MLV or VL30) produce viral particles at high titer. Furthermore, it appears that the REV-A sequence ranging from nucleotides 265 to 578 is able to enhance the encapsidation of the viral RNAs and

consequently the higher viral titer in comparison with the control vector pEMCV-CBTV comprising the EMCV IRES site. In comparison with the same control vector, the dicistronic retroviral vector pREV HW-3 comprising REV-A IRES sequence 265-578, is significantly more efficient in transducing reporter genes *in vitro* in the human cell line Dev, derived from a human primary tumor of neuroectodermal origin, whose cells behave like pluripotent stem cells. The specification further discloses that the expression of the reporter genes was unaffected by the differentiation state of these Dev cells.

The above evidence has been noted and considered. However it can not be reasonably extrapolated to the instant claimed invention, which when read in light of the specification is drawn to a pharmaceutical composition and a method for treating and preventing diseases such as, cystic fibrosis, hemophilia A or B, cancer, AIDS, cardiovascular diseases among others (See specification, pages 12-14). The nature of the instant claims lies within the realm of gene therapy art.

The specification is not enabled for the **use** of the claimed pharmaceutical composition and a treatment or preventing method as claimed, because at the effective filing date of the instant application the art of gene therapy was immature and highly unpredictable. In a meeting report on a workshop for gene therapy and translational cancer research (Clin. Cancer Res. 5:471-474, 1999), Dang et al. noted that further advancement in all fields including, gene delivery, gene expression, immune manipulation, and the development of molecular targets is needed to make gene therapy a reality. They further cited the findings of the Orkin-Motulsky Committee

(commissioned by the NIH director) which found that human gene therapy is an immature science with limited understanding of gene regulation and disease models for preclinical studies (First paragraph, page 471). Dang et al. pointed out several factors limiting an effective human gene therapy, including, sub-optimal vectors, the lack of long term and stable gene expression, and most importantly the efficient gene delivery to target tissues (last paragraph, page 474).

The breadth of the claims encompasses the use of a vector, a viral particle generated from a viral vector, a cell comprising a vector or a viral particle of the instant application to treat various diseases. However, the specification fails to provide sufficient guidance, direction and examples demonstrating the efficacy of the claimed pharmaceutical composition to treat or prevent any disease, and said composition is not even tested in any animal model of any disease for its therapeutic or prophylactic effects. With regard to the composition comprising the vector or viral particles of the present invention, the specification does not teach a skilled artisan that said composition could produce therapeutic or prophylactic proteins/polypeptides in a sufficient amount for a sufficient period of time *in vivo,* to yield desirable treatment results for a patient. This is a relevant issue since sub-optimal vector continues to be one of several factors limiting the effectiveness of gene therapy as mentioned above. In a review on gene delivery systems (both viral and non-viral vectors), Wivel and Wilson (Methods of gene delivery, Hematol. Oncol. Clin. North Am. 12:483-501, 1998) stated that "One of the major challenges still confronting the field is the design of more efficient vectors. The gene delivery systems being used today will undoubtedly be seen as

crude when compared with future developments. It is unlikely that there will ever be a universal vector, but rather there will be multiple vectors specifically designed for certain organ sites and certain diseases.... It will be necessary to do much more fundamental research in cell biology, virology, immunology, and pathophysiology before vectors can be significantly improved." (pages 498-499 in Summary section).

The breadth of the claims encompasses the expression of any and all genes of interest by the claimed composition in a host for treatment purposes. However, Eck and Wilson (Gene-based therapy, 1996) addressed several specific factors that complicate *in vivo* gene transfer and expression which result in therapeutic effects. These include, the fate of delivering vectors, the fraction of vectors taken up by the target cell population, the rate of vector degradation, the level of mRNA produced, the stability of the protein produced, the protein's compartmentalization within the cell or its secretory fate (Column 1, page 82). Even for localized administration of vectors, the above factors differ dramatically based on the protein being produced, and the desirable therapeutic effect being sought. Therefore, the level of gene expression, its duration, and its *in vivo* therapeutic effects are not always predictable, and hence without any *in vivo* examples provided by the instant application, it would have required undue experimentation for a skilled artisan to use the claimed invention.

The breadth of claim 37 encompasses any and all routes of administering a therapeutically effective quantity of a vector, a viral particle, a transformed or transfected cell of the prevent invention in a patient for treating or preventing a genetic disease, a cancer or an infection disease. The specification does not provide sufficient

guidance demonstrating that the pharmaceutical composition, in the forms of a vector or viral particles, can be delivered to target cells in an effective amount by any delivery means into a patient to obtain therapeutic or prophylactic effects. Particularly, in the presence of a competent immune response of a patient against most viral vectors. Vector targeting *in vivo* to desired tissues, organs continues to be unpredictable and inefficient. This is supported by numerous teachings available in the art. For examples, Miller & Vile (FASEB 9:190-199, 1995) reviewed the types of vectors available for *in vivo* gene therapy, and concluded that ""for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances .... Targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1). Deonarain (Exp. Opin. Ther. Patents 8:53-69, 1998) indicated that one of the biggest problems hampering successful gene therapy is the "ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time" (page 53, first paragraph). Deonarain also reviewed new techniques under experimentation in the art which show promise, but is currently even less efficient than viral gene delivery (see page 65, first pargraph under Conclusion section). Verma & Somia (Nature 389:239-242,1997) reviewed various vectors known in the art for use in gene therapy and the problems which are associated with each and clearly indicated that at the time of the claimed invention resolution to vector targeting had not been achieved in the art (see the entire article). Verma & Somia discussed the role of the immune system in inhibiting the efficient targeting of

viral vectors such that efficient expression is not achieved (see page 239, and second and third columns of page 242). The specification fails to teach one of skilled in the art how to overcome the unpredictability for vector targeting *in vivo*, such that an efficient and effective amount of therapeutic gene transfer could be achieved by any mode of delivery. Therefore, it would have required undue experimentation for a skilled artisan to practice the claimed invention.

With regard to the use of a pharmaceutical composition comprising of a cell comprising a vector or viral particles of the instant application for therapeutic purposes, there are similar and additional gene therapy hurdles that one skilled in the art would encounter. These include, the fate of such therapeutic cells once they are delivered into a subject, the duration and expression levels of transgenes of interest in the recombinant cells, and most importantly, the host immune responses against the administered therapeutic cells. It is already well known in the art that a major problem in cell transplantation is rejection of transplanted cells by the host. The instant claims would encompass the use of allogeneic, xenogeneic, as well as autologous genetically modified cells.

At the effective filing date of the instant application, cell transplantation therapies with genetically altered cells to treat diseases and disorders are neither routine nor predictable. As an example, regarding to the utilization of mesenchymal stem cells for human gene therapy, Gerson (Nature Med. 5:262-264, 1999) indicated many questions that need to be addressed, such as, "What is the minimum proportion of donor mesenchymal stem cells required to affect a long-lasting therapeutic response?", "Will.

transplantation of mesenchymal stem cells from a marrow harvest or from culture-expansion be sufficient to treat other diseases?", "Can culture-expanded mesenchymal stem cells substitute for fresh marrow allografts in the correction of genetic disorders of the mesenchyme?", "To which host tissues do infused mesenchymal stem cells home, proliferate and differentiate, and using which regulatory signals?", "Can mesenchymal stem cells be used effectively for gene transfer and gene deliver?", "Is systemic infusion optimal or is infusion into a target organ required?" (column 1, second paragraph, page 264). Similar questions and concerns would be raised for the utilization of other genetically modified cells encompassed by the breadth of the claimed pharmaceutical composition in the instant application for therapeutic or prophylactic purposes.

Accordingly, due to the lack of guidance presented in the specification regarding to the use of the claimed pharmaceutical composition and a method of treatment or preventing a genetic disease, a cancer or an infection disease, the absence of working examples, the breadth of the claims, and the unpredictability of the gene therapy art, it would have required undue experimentation for one skilled in the art to make and use the claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on January 05, 2001 in Paper No. 11 (pages 15-20) have been fully considered.

Applicants mainly argued that clinical benefits from gene therapy have been clearly demonstrated with a variety of vectors, therapeutic genes, administration routes

and applications on the basis of various studies summarized in a meeting report of Dang et al., as well as post-filing articles of Kay et al. (Nature genetics 24:257-261, 2000), Isner et al. (J. Clin. Invest. 103:1231-1236, 1999), Khuri et al. (Nature Medicine 6:879-885, 2000) and Cavazzana-Calvo et al. (Science 288:669-672, 2000). Therefore the instant specification is enabled for the pharmaceutical composition and a method of treatment as claimed. Examiner respectfully finds Applicants' arguments to be unpersuasive for the following reasons. First, it is noted that the effective filing date of the present application, the attainment of therapeutic effect via gene therapy was highly unpredictable as supported by Dang et al. Even in 1999, Dang et al. stated that ""This workshop reviewed some recent advances in gene delivery, gene expression, immune manipulation, and the development of molecular targets and stressed that all of these fields will need further advancement **to make gene therapy a reality** (page 471, column 1, first paragraph, last sentence, Clin. Cancer Res. 5:471-474, 1999). Clearly, at the effective filing date of the present application, achieving therapeutic effects via gene therapy was not routine, and given the lack of any guidance provided by the present application, it would have required undue experimentation for a skilled artisan to make and use the present invention. Especially, in light of the contemplated scope for **treating** and **preventing** any and all genetic diseases, any and all cancers and any and all infectious diseases without any factual evidence indicating the therapeutic effectiveness of the claimed pharmaceutical composition. Secondly, there is no correlation between the positive results obtained from the post-filing arts and various studies summarized in the article of Dang et al. in 1999 with the therapeutic results

contemplated by Applicants, because the starting materials as well as processing steps for achieving the positive results in those cited studies are not the same and they are not taught or supported by the instant specification. Thirdly, given the breadth of the instant claims, Applicants have not addressed how to overcome issues such as the lack of a long term and stable expression of transgene *in vivo* (absolutely essential for achieving prophylactic effects or preventing any disease), the adverse host immunological reactions that limit the effectiveness of targeted gene delivery, as well as the lack of an efficient gene delivery to desired cells or organs *in vivo* known in the art, such that therapeutic results contemplated by Applicants could be obtained.

Accordingly, claims 22, 23 and 37 are rejected under 35 U.S.C. 112, first paragraph for the reasons set forth above.

Amended claim 19 and new claims 40 and 41 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for an isolated cell comprising the vector of claim 8 or a viral particle generated from said vector and an *in vitro* method of expressing one or more genes of interest into pluripotent cells comprising the step of transfecting or infecting said pluripotent cells with the vector of claim 8 or a viral particle generated from said vector or a pharmaceutical composition comprising the same, does not reasonably provide enablement for the same *in vivo* cell and the same method *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

Claim 19 is drawn to a cell comprising a vector or infected with a viral particle generated from a viral vector according to claim 8. Claims 40 and 41 are drawn to a method for expressing one or more genes of interest into pluripotent cells, comprising the step of transfecting or infecting said pluripotent cells with a vector or a viral particle generated from a viral vector according to claim 8 or a pharmaceutical composition prepared from said vector or viral particle; the same method wherein said pluripotent cells are of the central nervous system.

The specification is not enabled for the instant broadly claimed invention. The breadth of the claim encompasses both *in vitro* and *in vivo* methods for transfection or infection of pluripotent cells and *in vitro* and *in vivo* cells comprising a vector or a viral particle of the instant invention. When read in light of the specification, the sole intended use for the *in vivo* cell or *in vivo* transfection or infection of pluripotent cells using a vector or a viral particle of the instantly claimed invention is for treatment purposes. The specification fails to provide any guidance demonstrating the infection of any pluripotent cells *in vivo* using a vector or viral particle of the instantly claimed invention comprising to yield any therapeutic effects contemplated by Applicants. The specification has not set forth conditions, parameters for one skilled in the art to overcome all the hurdles and limitations outlined in the rejection of claims 22, 23 and 37 above to practice the claimed invention without undue experimentation.

Accordingly, due to the lack of guidance provided by the specification regarding to the *in vivo* scope of the instant claims, the breadth of the claims, the state and the

unpredictability of the gene therapy art, it would have required undue experimentation for one skilled in the art to make and use the broadly claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on January 05, 2001 in Paper No. 11 (pages 25-26) have been fully considered.

With reference to the post-filing art of Derrington et al. (Human gene therapy 10:1129-1138, 1999), Applicants argued that it was known in 1992-1995 that neural precursor cells can be isolated, expanded *in vitro* and grafted back into the brains of host animals with their neuronal and glial progeny developing appropriately in the host brains. Applicants further quoted a statement from the same authors who stated that "the capacity of CNS-derived multipotent precursors to integrate functionally within a host brain suggests that transplantation of neural precursor cells that have been genetically engineered to produce molecules of therapeutic potential may provide a long term strategy to modulate the consequences of neurogenetic diseases or neural degeneration in the brain" (page 26). Therefore, the present application provides more than an adequate description to permit the practice of the claimed method. Examiner respectfully finds Applicants' argument to be unpersuasive. It is noted that the *in vivo* scope of the methods as claimed falls within an *in vivo* gene therapy method, not an ex *vivo* gene therapy method. Additionally, with regard to the quoted statement Examiner noted that transplantation of neural precursor cells that have been genetically engineered to produce molecules of therapeutic potential **may** provide a long term to

modulate the consequences of neurogenetic diseases or neural degeneration in the brain. With respect to the unpredictability for achieving therapeutic results via gene therapy (both *in vivo* and *ex vivo*) because of the various factors known in the art to limit the effectiveness of gene therapy (discussed in the rejection of claims 22, 23 and 37) along with the lack of any factual evidence indicating that the instant claimed invention can yield any therapeutic effects contemplated by Applicants, it would have required undue experimentation for a skilled artisan to make and use the full scope of the instant claims.

Accordingly, claim 19 and new claims 40 and 41 are rejected under 35 U.S.C. 112, first paragraph for the reasons set forth above.

Claim 39 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention for the same reasons already stated in the previous Office Action in Paper No. 9 (pages 13-15).

The claim is directed to a method for producing a transgenic animal, comprising the step of integrating into the genome of said animal a vector for the expression of one or more genes of interest according to claim 8.

The specification is not enabled for the instant claimed invention, because at the effective filing date of the instant application, the art of transgenesis is highly unpredictable. The specification fails to provide any guidance or direction or examples

regarding to the production of any type of transgenic animal, let alone, the harvest of polypeptides of interest from the biological fluids of said transgenic animal. The specification merely recites that "The techniques for generating these transgenic animals are known. The polypeptide of interest may be recovered in a conventional manner, for example, from the biological fluids (blood, milk and the like) of the animal (page 18, lines 20-24). The specification fails to set forth any parameters or conditions for the generation of any kind of transgenic animal, for examples, therapeutic gene constructs available for use, promoters used for the expression of therapeutic gene products into the biological fluids of a transgenic animal, among others. Mullins et al. (J. Clin. Invest. 98:S37-S40, 1996) have noted the positional effects due to the random integration of exogenous DNA into chromosomal DNA have major consequences on the expression of the transgene, including loss of cell specificity, inappropriately high copy number-independent expression and complete silencing of the transgene (column 2, first paragraph, page S37). Because of the unpredictable nature of random transgene integration and positional effects, it would be extremely difficult to predict successful transgene transfer and its expression in any transgenic animal. The breadth of the instant claim encompasses any and all transgenic animals. However, it is well known in the art, that the production of transgenic animals other than mice is undeveloped. This is because ES cell technology is generally limited to the mouse system, at present, and that only "putative" ES cells exist for other species. This observation is supported by Seamark (Reprod. Fert. Dev. 6:653-657, 1994) by reporting that totipotency for ES cell technology in many livestock species has not been demonstrated (Page 6, Abstract).

Likewise, Mullins et al. stated that "although to date chimeric animals have been generated from several species including the pig, in no species other than the mouse has germline transmission of an ES cell been successfully demonstrated." (Page S38, column 1, first paragraph). Mullins et al. further stated that "a given construct may react very differently from one species to another." (Page S39, summary). Wall (Theriogenology 45:57-68, 1996) supported this observation by stating that "Our lack of understanding of essential genetic control elements makes it difficult to design transgenes with predictable behavior." (Page 61, last paragraph) and "transgene expression and the physiological consequences of transgene products in livestock are not always predicted in transgenic mouse studies." (Page 62, first paragraph).

Accordingly, due to the lack of guidance provided by the specification regarding to the issues set forth above, the breadth of the claims, the state and the unpredictability of the transgenic art, it would have required undue experimentation for one skilled in the art to make and use the instantly claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on January 05, 2001 in Paper No. 11 (pages 23-25) have been fully considered.

Applicants mainly argued that successful transgenic experiments have been reported in various publications, for examples Mullins et al., U.S. Patent No. 5,175,383; U.S. Patent No. 4,736,866 and 5,175,384). Applicants further argued that the state of the art establish that transgenesis had a reasonable expectation of success in a wide

variety of species at the effective filing date of the present application. Examiner respectfully finds Applicants' arguments to be unpersuasive for the following reasons. Firstly, there is no correlation between the successful transgenic animals made in the cited publications with a transgenic animal whose genome comprises a vector for the expression of one or more genes of interest according to claim 8 of the present invention. Secondly, on the contrary to Applicants' assessment of the state of transgenesis at the effective filing date of the present application, Mullins et al. (J. Clin. Invest. 98:S37-S40, 1996) have noted the positional effects due to the random integration of exogenous DNA into chromosomal DNA have major consequences on the expression of the transgene, including loss of cell specificity, inappropriately high copy number-independent expression and complete silencing of the transgene (column 2, first paragraph, page S37). Because of the unpredictable nature of random transgene integration and positional effects, it would be extremely difficult to predict successful transgene transfer and its expression in any transgenic animal. Additionally, it is well known in the art, that the production of transgenic animals other than mice is undeveloped as evidenced by the teachings of Mullins et al., Seamark and Wall as discussed above. Thirdly, Applicants have not provided any factual evidence indicating otherwise.

Accordingly, claim 39 is rejected under 35 U.S.C. 112, first paragraph for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 8-19, 22-23, 25-39 and 42-50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claims 8, 25, 31 and their dependent claims, it is unclear what is encompassed by the phrase "isolated from the 5' end of the genomic RNA of a type C retrovirus" because one does not know where is the 3' end boundary of the nucleotide sequence. Therefore, the metes and bounds of the claim can not be clearly determined. Additionally there is no clear and exact definition for the 5' end of the genomic RNA of a type C retrovirus in the specification. Furthermore, the phrase "the DNA equivalent of said genomic RNA" is also unclear. What exactly is it? Clarification is requested.

In claim 12, the phrase "a third gene of interest" is unclear. Where is the second gene of interest? Clarification is requested.

In claim 48, the phrase "further comprises a first gene of interest" is unclear. This claim is dependent on a vector of claim 12, in which two genes of interest are already present, and therefore an additional gene of interest is not a first gene of interest. Additionally, it is unclear where is the position of this additional gene of interest with respect to the positions of the first two genes of interest? The metes and bounds of the claim can not be clearly determined. Clarification is requested.

The term "substantially homologous" in claims 14, 16, 28. 29, 34, 35, 44 and 45 is a relative term which renders the claim indefinite. The term "substantially homologous" is not defined by the claim, the specification does not provide a standard

for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Therefore, the metes and bounds of the claims can not be clearly determined.

In claim 38 it is unclear what is encompassed by the phrase "by the recombination route". Which recombination route that Applicants refer to? Clarification is requested.

In claim 39, the claimed method lacks all essential steps required for the product of a transgenic animal whose genome comprises a vector of claim 8 for the expression of one or more genes of interest.

Claim Rejections - 35 USC § 102

Claims 8, 9, 10, 12, 18, 19, 22, 25 and 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Berlioz et al. (J. Virol. 69:6400-6407, 1995) for the reasons set forth in the previous Office Action in Paper No. 9 (pages 23-25).

With respect to claim 22 drawn to a pharmaceutical composition comprising the vector, viral particle or a transformed or transfected cell of the present invention in combination with a pharmaceutically acceptable vehicle, the intended use of a pharmaceutical composition is not given any patentable weight in view of prior art.

With respect to the enabled scope of the instant claimed invention, Berlioz et al. disclosed the preparation of monocistronic and dicistronic plasmid DNA constructs comprising the rat VL30 region of the Harvey murine sarcoma virus (a member of the type C retrovirus family) leader, and demonstrated that the rat VL30 region serves as an

IRES site and efficiently directs the expression of a 3'cistron *in vitro* and *in vivo* (See Figs. 1, 3, 5 and 7). Berlioz et al. also disclosed the construction of a dicistronic MLV-derived retroviral vector, pVL-CBT2, comprising the VL30 sequence (nucleotides 205 to 794) inserted between phosphatase and neomycin genes. Recombinant viral particles that were generated from the transient transfection of pVL-CBT2 viral vector into ecotropic GP-E+86 helper cells were used to infect NIH-3T3 cells to demonstrate that the 5' VL30 sequence provides an IRES for efficient translation of the neomycin gene positioned downstream of said sequence, and for packaging of RNA into MLV virions (See Fig. 8 and column 2, page 6405). Since sterile and filtered water or culture media, in which vectors, viral particles and transfected cells are normally dispersed in, are considered to be pharmaceutically acceptable carrier (See page 6401, col.2, last paragraph), the teachings of Berlioz et al. met all the limitation of the claims. Therefore, the reference anticipates the claimed invention.

Response to Arguments

Applicants' argument related to the above rejection in the Amendment filed on January 05, 2001 in Paper No. 11 (page 32) has been fully considered.

Applicants argued that the scope of the claims has been restricted to the use of a nucleotide sequence isolated from the 5' end of the genomic RNA of a REV or MSV virus, and that this limitation renders the claimed invention novel over Berlioz et al. Examiner would like to point out that the VL30 region was isolated from the 5' end of the Havery murine sarcoma virus, belonging to the type C retrovirus family, which is also an

MSV (murine sarcoma virus). Therefore, the teachings of Berlioz et al. still meet the limitations of the claims as recited.

Accordingly, claims 8, 9, 10, 12, 18, 19, 22, 25 and 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Berlioz et al. (J. Virol. 69:6400-6407, 1995) for the reasons set forth above.

Claim Rejections - 35 USC § 103

Claims 8, 10, 11, 12, 47, 17, 22, 23, 38, 40-41 and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Berlioz et al. (U.S. Patent No. 5,925,565) in view of Berlioz et al. (J. Virol. 69:6400-6407, 1995) for the reasons already stated in the previous Office Action in Paper No. 9 (pages 26-27).

Regarding to claims 22 and 23, the intended use of a pharmaceutical composition is not given any patentable weight in view of prior art.

With respect to the enabled scope of the instant claimed invention, Berlioz et al. disclosed a polycistronic vector (including retroviral vector), a viral particle, an isolated cell comprising the recombinant vector or viral particle, and a method for incorporating a DNA encoding a protein of interest into a cell *in vitro*, that meet the limitations recited in the claims, except that the IRES site and the encapsidation region of the VL30 murine retrotransposon is not explicitly stated to be derived from the 5' end of the genomic RNA of a type C retrovirus (See claims and Figs. 2 and 3). Berlioz et al. taught relevant information regarding to limitations recited for claims 8, 10, 11, 47 (see pVL-CBT2-E+ construct and col. 7, line 60 continues to first paragraph of col. 8); claim 17 (see col. 5,

line 32 continues to line 6 in col. 6); claims 22and 23 (see col. 7, lines 41-45); claim 38 (see col. 5, lines 1-13 and col. 7, lines 1-14), claims 12 and 48 (see Fig. 1C, middle construct). In an earlier reference (J. Virol. 69:6400-6407, 1995), Berlioz et al. disclosed the identical VL30 region can be obtained from the Harvey murine sarcoma virus (HaMSV) leader, and HaMSV is a member of the type C retrovirus family (See page 640). Although Berlioz et al. (U.S. Patent No. 5,925,565) do not specifically teach expressing one or more genes of interest into pluripotent cells *in vitro*, particularly those of central nervous system, the scope of claim 19 in the issued patent encompasses such cells, and it is within the scope of an ordinary skilled in the art to do so with a predictable expectation of success. Therefore, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' argument related to the above rejection in the Amendment filed on January 05, 2001 in Paper No. 11 (page 33) has been fully considered.

Applicants mainly argued that the prior art does not disclose a vector, viral particle or cell comprising a nucleotide sequence isolated from the 5' end of the genomic RNA of a REV or MSV virus and methods using thereof. Examiner would like to point out that the VL30 region was isolated from the 5' end of the Havery murine sarcoma virus, belonging to the type C retrovirus family, which is also an MSV (murine sarcoma virus). Therefore, the teachings of Berlioz et al. (U.S. Patent No. 5,925,565) in

view of Berlioz et al. (J. Virol. 69:6400-6407, 1995) render the instant claimed invention obvious.

Accordingly, claims 8, 10, 11, 12, 47, 17, 22, 23, 38 and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Berlioz et al. (U.S. Patent No. 5,925,565) in view of Berlioz et al. (J. Virol. 69:6400-6407, 1995) for the reasons already stated above.

Examiner would like to note that part of the teachings of Berlioz et al. (U.S. Patent No. 5,925,565) appear in the article of Torrent et al. (Human gene therapy 7:603-612, 1996).

Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Berlioz et al. (J. Virol. 69:6400-6407, 1995) in view of Dirks et al. (U.S. Patent No. 6,060,273) for the same reasons stated in the previous Office Action in Paper No. 9 (pages 27-28).

Berlioz et al. disclosed the construction of a dicistronic MLV-derived retroviral vector, pVL-CBT2, comprising the VL30 sequence (nucleotides 205 to 794) of the Harvey murine sarcoma virus (a member of the type C retrovirus family) leader inserted between phosphatase and neomycin genes. Berlioz et al. further taught that the 5' VL30 sequence functions as an IRES for efficient translation of the neomycin gene positioned downstream of said sequence, and for packaging of RNA into MLV virions (See Fig. 8 and column 2, page 6405). The disclosed vector of Berlioz et al., however, does not comprise a gene encoding for a product selected from factor VIII, factor IX, the CFTR

protein, dystrophin, insulin, alpha-, beta-, gamma interferon or an interleukin. However, Dirks et al. disclosed multicistronic expression units in which the cistrons comprise genes encoding factor VIII, creatine kinase, haemoglobin, scatter factor among others (See claim 9).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify a dicistronic MLV-derived retroviral vector of Berlioz et al. with the teachings of Dirk et al. by substituting a gene encoding phosphatase with one encoding factor VIII. The motivation for one of skill in the art to carry out such modification is to produce recombinant factor VIII subunits for preparation of pharmaceutical composition to treat blood disorders. Thus, the claimed invention was *prima facie* obvious in the absence of evident to the contrary.

Response to Arguments

Applicants' argument related to the above rejection in the Amendment filed on January 05, 2001 in Paper No. 11 (page 34-35) has been fully considered.

Applicants mainly argued that the limitation of claim 17 to a recombinant vector employing a nucleotide sequence isolated from the 5' end of the gnomic RNA of a REV or MSV virus renders the claimed invention patentable over the state of the art. Examiner would like to point out that the VL30 region was isolated from the 5' end of the Harvey murine sarcoma virus, belonging to the type C retrovirus family, which is also an MSV (murine sarcoma virus). Therefore, the teachings of Berlioz et al. (J. Virol.

69:6400-6407, 1995) in view of Dirks et al. (U.S. Patent No. 6,060,273) render the instant claim obvious.

Accordingly, claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Berlioz et al. (J. Virol. 69:6400-6407, 1995) in view of Dirks et al. (U.S. Patent No. 6,060,273) for the reasons stated above.

Upon further careful consideration, following is a new ground of rejection.

Claim Rejections - 35 USC § 112

Claims 14, 16, 50, 28, 29, 34, 35 and 44-45 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a vector or a retroviral vector for the expression of one or more genes of interest comprising a nucleotide sequence that is identical to SEQ ID NO:1 or SEQ ID NO:2 : (i) starting at nucleotide 1 and ending at nucleotide 578, (ii) starting at nucleotide 265 and ending at nucleotide 578 or (iii) starting at nucleotide 452 and ending at nucleotide 578; and methods of making the same, does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are drawn to the retroviral vector and the vector of the present invention wherein the nucleotide sequence isolated from the 5' end of the genomic RNA of REV or from the equivalent DNA of said genomic RNA is substantially homologous or identical to SEQ ID NO:1 or SEQ ID NO:2 and methods of making the same.

The specification is not enabled for the instant broadly claimed invention because apart from the disclosure of SEQ ID NO:1, SEQ ID NO:2 (nucleotides 1-578), and the two fragments of SEQ ID NO:2 (a fragment starting from nucleotide 265 to nucleotide 578, the other fragment starting from nucleotide 452 to nucleotide 578), the present specification fails to provide sufficient guidance for a skilled artisan on how to make and use nucleotide sequences that are substantially homologous to SEQ ID NO:1, SEQ ID NO:2 and its two fragments. When read in light of the specification, the breadth of these claims encompasses any and all fragments of at least 100 nucleotides in length, any and all modifications (including deletion, insertion or substitution) of SEQ ID NO:1 and SEQ ID NO: 2 (see specification, page 7, lines 20-27; page 8, lines 2-7). As is well recognized in the art, any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. The present disclosure offers no guidance as to which regions of the disclosed sequences would be tolerant of alteration and which would not, which "particular" nucleotide changes (substitution, deletion or insertion) at which position and at which combinations, such that the variant nucleotide sequences could still possess the desired functions, for this instance the IRES and encapsidation functional properties. There is a high degree of unpredictability associated with the make and use of the claimed embodiment. This situation is similar to the unpredictability in making of a protein or a peptide variant having the desired functional property. In discussing peptide hormones, Rudinger has stated that "The significance of particular amino acids and sequences for different aspects of biological activity can not be predicted a priori but must be

determined from case to case by painstaking experimental study (Page 6, first sentence of Conclusions *In* J.A. Parsons, ed. "Peptide hormones", University Park Press, 1976). This unpredictability is further underscored by the fact that the relationship between the sequence of a peptide and its tertiary by the fact that the relationship between the sequence of a peptide and its tertiary structure (or its activity) is not well understood and is not predictable (Ngo et al., *In* K. Merz et al., ed. "The protein folding problem and tertiary structure prediction", Birkhauser, 1994, 491-495). Moreover, the physiological art is recognized as unpredictable (MPEP 2164.03). As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictablity of factors involved.

Accordingly, given the lack of guidance provided by the instant specification, the breath of the claims, and the unpredictability associated with the making and using of the sequence substantially homologous to SEQ ID NO:1 or SEQ ID NO:2, it would have require undue experimentation for a skilled artisan to make and use the instant broadly claimed invention.

Claim Rejections - 35 USC § 103

Claims 8, 10, 12, 48 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Berlioz et al. (U.S. Patent No. 5,925,565) in view of Berlioz et al. (J. Virol. 69:6400-6407, 1995) as applied to claims 8, 10, 11, 12, 47, 17, 22, 23, 38 and 48 above, and further in view of Sands et al. (U.S. Patent No. 6,136,566).

Claim 13 is drawn to the retroviral vector according to claim 48, in which the internal promoter region, the second gene of interest, the IRES site and the third gene of interest are in an opposite orientation relative to the retroviral 5' and 3' LTRs.

The teachings of Berlioz et al. (U.S. Patent No. 5,925,565 and J. Virol. 69:6400-6407, 1995) have been discussed above, particularly to Fig. 1C, middle construct. Although Beriloz et al. (U.S. Patent No. 5,925,565) teach that the vector of their invention comprising several expression cassettes and that these can be inserted in either orientation with respect to one another: in the same orientation or in the reverse orientation (col. 4, lines 13-18), Berlioz et al. do not specifically teach that the internal promoter region, the second gene of interest, the IRES site and the third gene of interest are in an opposite orientation relative to the retroviral 5' and 3' LTRs. However, Sands et al. teach in constructing their retroviral vectors, the direction of transcription of the selectable marker cassette should be opposite to the direction of the normal transcription of the retrovirus. The reason for this organization is that the transcription elements such as the polyadenylation signal, the splice sites and the promoter elements found in their various retroviral constructs interfere with the proper transcription of the retroviral genome in the packaging cell line, and this leads to the elimination or significantly reduction of retroviral titers (col. 10, lines 24-40).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the orientation of the internal promoter, IRES site and their operably linked genes of interest in the vector disclosed by Berlioz et al. to be in the opposite orientation to the retroviral 5' and 3' LTRs as taught by Sands et al., to avoid the potential reduction in retroviral titers. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusions

Claims 14-16, 49, 50, 26-30, 32-36, 38 and 42-46 are free of prior art. At the time of the instant invention, the prior art did not teach or fairly suggest a vector and methods of uses as claimed.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Karen Hauda, at (703) 305-6608.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Patsy Zimmerman, whose telephone number is (703) 308-0009.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1632.

Quang Nguyen, Ph.D.

DAVE T. NGUYEN
PRIMARY EXAMINER